



Wang, L., Lin, Y., Zhou, Y., Xie, H., Song, J., Li, M., Huang, Y., Huang, X., & Mann, S. (2019). Autonomic Behaviors in Lipase-Active Oil Droplets. *Angewandte Chemie - International Edition*, 58(4), 1067-1071. <https://doi.org/10.1002/anie.201812111>

Peer reviewed version

License (if available):
Other

Link to published version (if available):
[10.1002/anie.201812111](https://doi.org/10.1002/anie.201812111)

[Link to publication record in Explore Bristol Research](#)
PDF-document

This is the accepted author manuscript (AAM). The final published version (version of record) is available online via Wiley at <https://doi.org/10.1002/anie.201812111> . Please refer to any applicable terms of use of the publisher.

University of Bristol - Explore Bristol Research

General rights

This document is made available in accordance with publisher policies. Please cite only the published version using the reference above. Full terms of use are available:
<http://www.bristol.ac.uk/red/research-policy/pure/user-guides/ebr-terms/>

Autonomic Behavior in Lipase-active Oil Droplets

Lei Wang^{[a],†}, Youping Lin^{[a],†}, Yuting Zhou^[a], Hui Xie^[b], Jianmin Song^[b], Mei Li^[c],
Yudong Huang^[a], Xin Huang^{[a],*} and Stephen Mann^{[c],*}

^a MIIT Key Laboratory of Critical Materials Technology for New Energy Conversion and Storage, School of Chemistry & Chemical Engineering, Harbin Institute of Technology (HIT), Harbin, 150001, China.

^b State Key Laboratory of Robotics & Systems, HIT, Harbin, 150080, China.

^c Centre for Protolife Research & Centre for Organized Matter Chemistry, School of Chemistry, University of Bristol, Bristol, BS8 1TS, United Kingdom.

[†]These authors contributed equally to this work.

*E-mails: xinhuang@hit.edu.cn; s.mann@bristol.ac.uk.

Abstract

Developing self-fueled micro-reactor droplets with programmable autonomic behaviors provides a step towards smart liquid dispersions comprising motile microscale objects. Here we prepare aqueous suspensions of lipase-coated oil globules comprising a mixture of a triglyceride substrate (tributyrin, 1,2,3-tributylglycerol) and a low-density oil (polydimethylsiloxane, PDMS), and describe a range of active behaviors based on controlled enzyme-mediated consumption of individual droplets under non-equilibrium conditions. Encapsulation of the lipase-coated lipid/PDMS droplets into a model protocell as energy-rich sub-compartments is demonstrated as an internalized mechanism for activating protocell buoyancy. Taken together, our results highlight opportunities for the regulation of autonomic behavior in enzyme-powered oil droplets, and provide a new platform for increasing the functionality and energization of synthetic protocells.

Recent progress in the development of novel types of functional micro-compartmentalized ensembles (synthetic protocells) that are capable of mimicking rudimentary aspects of living cells has re-focused attention on the deep question of how inanimate systems can transition into living matter.^[1] Currently, membrane-bounded micro-compartments based on the self-assembly of fatty acids,^[2] phospholipids,^[3] synthetic polymers,^[4] amphiphilic inorganic nanoparticles,^[5] protein-polymer nanoconjugates (proteinosomes),^[6] as well as molecularly crowded

membrane-free coacervate micro-droplets^[7] have been exploited as synthetic chassis for the integration of a wide range of functional components and biochemical machinery. As a consequence, new types of synthetic protocells capable of cell-free gene expression,^[8] RNA catalysis,^[9] enhanced protein refolding,^[10] multi-compartmentalization,^[11] membrane growth, reproduction and division,^[12] phagocytosis^[13] and predatory behavior^[14] have been recently reported. Whilst these studies have advanced the design and construction of novel protocell models with bespoke structures, functions and behaviours, incorporating autonomic properties into these synthetic micro-architectures remains a considerable challenge.^[15] This raises the question of whether the unique dynamics of certain inanimate systems (“active matter”) could be used to model transitions to a life-like state in which information, chemical metabolism and self-organization are integrated and recursively exploited to generate a sustained flux of energy and exchange of matter. In this regard, recent studies on oleic acid-based vesicles or oil-in-water droplets prepared under non-equilibrium conditions have shown an astonishing range of life-like behaviours^[16] including movement^[17], division^[18], fusion^[19] and chemotaxis^[20] depending on the droplet composition, rate of interfacial molecular reactions, presence of Marangoni instabilities, and imbalance in surface tension initiated by symmetry breaking.

Inspired by these studies, herein we develop a facile method to produce enzyme-coated oil globules in water that are capable of a range of non-equilibrium active behaviors based on controlled enzyme-mediated consumption of the individual droplets. For this, we prepare stabilized aqueous suspensions of lipase-coated oil droplets comprising a mixture of a lipid triglyceride substrate (tributylin) and a low-density polydimethylsiloxane (PDMS) and initiate or impede lipase-mediated consumption of the droplets by judicious changes in temperature to control the rate of *in situ* substrate hydrolysis. We show that spontaneous uptake of the oil into the atrophied droplets can occur at low temperatures by transfer of new feedstock from non-lipase active stabilized tributyrin droplets or a floating layer of the triglyceride substrate. As a consequence, enzyme activity in the oil droplets is sustained under conditions of high reactivity when the net exchange associated with the rate of

substrate uptake and rate of lipase-mediated consumption reaches a steady state. Significantly, the lipase-mediated decrease in density of the lipid/PDMS droplets results in an increase in buoyancy, which can be subsequently offset by additional uptake of substrate uptake in the floating PDMS globules such that the droplets exhibit reversible vertical movement in a water column. We encapsulate the lipase-coated lipid/PDMS droplets within the water-filled interior of semi-permeable protein-polymer microcapsules as a step towards constructing a model protocell containing energy-rich sub-compartments capable of activating a buoyant force. Taken together, our results highlight opportunities for the regulation of autonomic behavior in enzyme-powered oil droplets, and provide a new platform for increasing the functionality and energization of synthetic protocells.

A dispersion of lipase-stabilized oil-in-water emulsion droplets was prepared at a range of temperatures by vigorously shaking a mixture of tributyrin and PDMS (9:1 v/v; 20 μ L) with an aqueous solution of lipase (200 μ L, 2.0 mg/mL) to give a water/oil volume fraction (ϕ_w) of 10. Oil droplets with diameters ranging from 10 to 60 μ m were produced under a range of conditions (Fig. 1a, Fig. S1). Confocal fluorescence microscopy images of the globules prepared with red fluorescent Rhodamine B isothiocyanate (RBITC)-labelled lipase and stained with oil-soluble green fluorescent (PFOPV, see SI Materials) showed the presence of green fluorescence throughout the droplets along with a red fluorescence protein membrane (Fig. 1b,c). The droplets remained non-aggregated for extended periods of time at temperatures between 0 and 60 $^{\circ}$ C and pH values between 2 and 10 (Fig. S2) and showed no change in mean diameter when incubated at 0 $^{\circ}$ C (Fig. S3). In contrast, oil droplets produced in the absence of lipase underwent complete coalescence within 5 min, indicating that the enzyme was required for stabilization of the oil droplet/water interface. Changes in ϕ_w had minimal effect on the formation of stable droplets provided that the water/oil ratio remained greater than 1.0. Lipase could also be used to prepare aqueous suspensions of oil droplets comprising 2-ethyl-1-hexanol (Fig. S4), and other enzymes such as lyticase could be employed to stabilize the tributyrin/PDMS droplets (Fig. S5). Significantly, optical microscopy (Fig. 1d), SEM

and AFM images (Fig. S6a,b) of the air-dried lipase-stabilized oil droplets showed the presence of an intact but wrinkled membrane associated with the collapsed microstructures. The thickness of the membrane was determined as 7.46 nm (Fig. S6c), which was consistent with the average molecular size of lipase in solution (6.78 nm) (Fig. S7). Taken together, the results indicated that the triglyceride/PDMS droplets were stabilized in water by the presence of a self-assembled lipase monolayer at the oil/water interface.

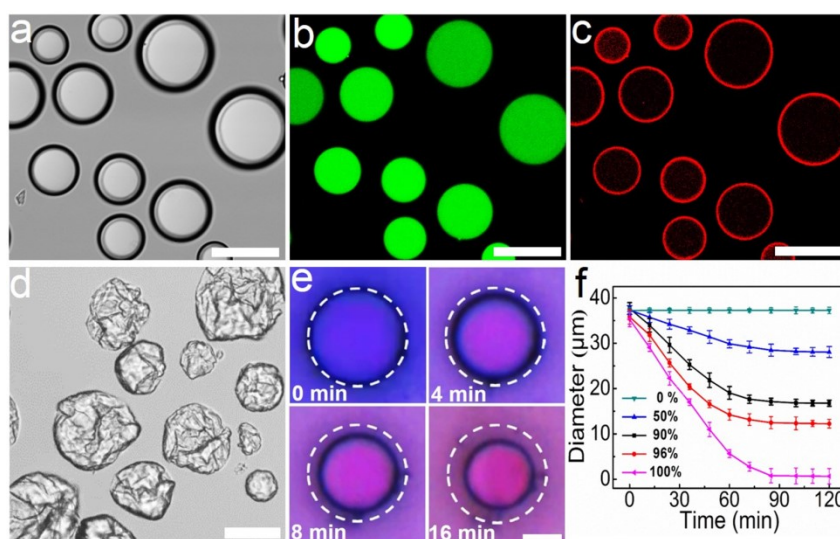


Figure 1. (a) Optical and (b,c) confocal fluorescence microscopy images of a suspension of lipid/PDMS droplets in water at 0 °C. The droplets are stabilized with RBITC-lipase and stained with the oil soluble dye PFOPV. (d) Optical microscopy images of dried lipase-coated droplets (oil phase, dichloromethane) showing collapsed microcapsules each with a wrinkled membrane. (e) Time-lapse optical microscopy images showing a single oil droplet (tributyrin/PDMS; 9:1 v/v) undergoing *in situ* lipase-mediated hydrolysis at 25 °C. The decrease in size associated with expulsion of glycerol and butyric acid to the external medium is accompanied by acidification of the continuous water phase from blue (pH ~6.5) to pink (pH ~3.2) within 16 min. (f) Plots showing changes in average diameter of lipase-coated oil droplets as a function of time at different volume ratios of tributyrin and PDMS at 25 °C. Scale bars, 50 μm (a-c), 15 μm (d) and 10 μm (e).

Increasing the temperature above 17 °C initiated lipase-mediated consumption of tributyrin within the lipid/PDMS droplets and expulsion of glycerol and butyric acid reaction products into the water phase (Fig. S8). As a consequence, the droplets decreased in size as the triglyceride was depleted and the surrounding aqueous phase became progressively acidified (Fig. 1e, S9-10, Movie S1-2). The degree of lipase-induced atrophy was modulated by adjusting the tributyrin/PDMS volume ratio

to produce oil droplets with a range of reduced diameters (Fig. 1f). In each case, interfacial catalysis ultimately resulted in lipase-coated enzymatically inactive PDMS droplets except for globules of pure tributyrin that were reduced to solid particles of lipase (100% atrophy). The atrophied droplets remained approximately spherical in shape, structurally intact and non-aggregated (Fig. S11-12). AFM force measurements indicated that the self-induced consumption of lipid droplets caused droplet shrinkage, which increased the measured force from ~ 0.12 to ~ 1.47 MPa, indicating that the membrane became more compact after contraction (Fig. S13a). In addition, line profiles obtained using scanning ion conductance microscope displayed an increase in the surface roughness of the atrophied droplets (Fig. S13b), confirming that wrinkling of the intact lipase membrane occurred as the globules decreased in size. This was consistent with UV spectra recorded on the aqueous continuous phase (Fig. S14), which showed no changes in the concentration of free lipase as the droplets contracted in size, indicating that negligible levels of lipase were released into the bulk solution as the oil globules were consumed. Significantly, *in situ* hydrolysis of the globules resulted in a decrease in droplet density (PDMS, 0.972 g/mL; tributyrin, 1.032 g/mL) due to a progressive enrichment in PDMS such that the atrophied droplets became buoyant in the aqueous suspension.

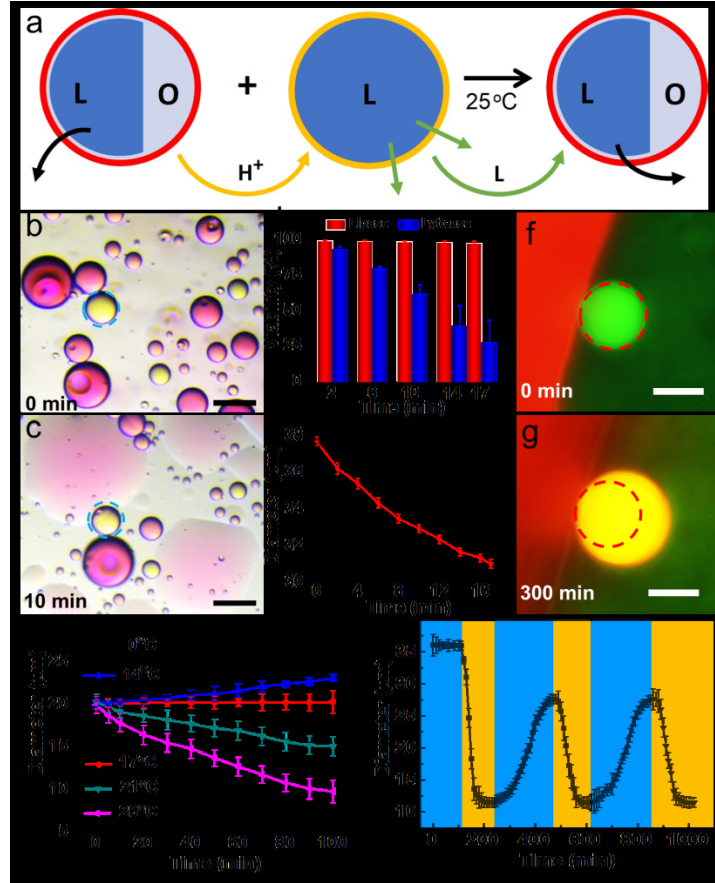


Figure 2. (a) Graphic depicting steady-state preservation of enzyme activity and droplet size at 25 °C for a lipase (red)-coated tributyrin (L; dark blue)/PDMS (O; light blue) droplet suspended in water in the presence of tributyrin globules initially stabilized by a pH-sensitive lyticase membrane (orange). Self-induced hydrolysis of tributyrin releases glycerol and butyric acid (curved black arrow) into the solution and produces protons (curved orange arrow) that disassemble the lyticase-stabilized globules, thereby releasing new feedstock for turnover by the lipase-active droplets (green arrows). (b,c) Time-lapse optical microscopy images of an aqueous suspension consisting of a binary population of lipase-coated tributyrin/PDMS droplets (yellow) and lyticase-stabilized tributyrin droplets (pink) at 25 °C. Acid-induced release of tributyrin from the lyticase-stabilized droplets is observed within 17 minutes (large pink patches in b). Scale bars, 50 μm. Pink and yellow dyes are Nile red and PFOPV, respectively. (d) Population dynamics in a binary community of lipase-active (red bars) and lyticase-stabilized (blue bars) oil droplets; the number of lipase-coated droplets (viability%) remains unchanged whilst the population of lyticase-stabilized globules is reduced by ca. 75% over a period of 17 min. (e) Plots showing time-dependent decreases in the diameter of lipase-active droplets in the presence (black) or absence (red) of lyticase-stabilized tributyrin droplets at 25 °C. (f-g) Time-lapse fluorescence microscopy images showing the uptake of tributyrin into a single atrophied lipase-stabilized droplet (tributyrin/PDMS 9:1 v/v) locating under a floating layer of the triglyceride at 0 °C. The droplet increases in diameter from ~27 to ~37 μm over a period of 300 min. Red and green fluorescence in f (0 min) corresponds to Nile red in the floating tributyrin layer and FITC-lipase in the droplet, respectively. Yellow coloration in g (300 min) arises from the superimposition of red and green fluorescence associated with uptake of Nile red-stained tributyrin into the lipid droplet. The red

fluorescence intensity associated with the droplet at 300 min was *ca.* 140 times higher than that recorded at 0 min. Scale bar, 20 μ m. **(h)** Plot showing time-dependent changes in the diameter of partially atrophied lipase-stabilized oil droplets in the presence of excess tributyrin. The droplets are either reconstituted with additional feedstock or further atrophied depending on the temperature-dependent balance between the rates of tributyrin uptake and lipase-mediated *in situ* hydrolysis. A steady state growth/decay process is observed at ~ 17 $^{\circ}$ C. **(i)** Plot showing time-dependent oscillations in the mean diameter of lipase-active oil droplets subjected to temperature-directed changes in the relative rates of substrate uptake and consumption. The temperature was cycled between 0 $^{\circ}$ C (blue regions) and 25 $^{\circ}$ C (yellow regions) to switch off and on the enzyme, respectively. Experiments were repeated 5 times with >100 droplets counted each time.

Restoration of the atrophied oil droplets and steady-state preservation of their lipase activity under conditions of high reactivity (25 $^{\circ}$ C) was achieved by offsetting the hydrolysis-induced depletion in triglyceride concentration by *in situ* sequestration of new feedstock *via* pH-mediated inter-droplet transfer (Fig. 2a). This was achieved by preparing a binary population of lipase-active tributyrin/PDMS (9:1 v/v) droplets and pH-sensitive lyticase-stabilized tributyrin droplets (Fig. S15) at 25 $^{\circ}$ C and monitoring the two populations by optical microscopy (Fig. 2b-c). Acidification of the solution to a pH value of *ca.* 3.5 by the release of butyric acid during consumption of the lipase-active globules resulted in the depletion of the lyticase-containing droplet population over 17 min (Fig. 2d, Movie S3). Sequestration of the released tributyrin by the lipase-coated globules was sufficient to offset continued lipase activity so that steady states in droplet size and reactivity were achieved at 25 $^{\circ}$ C as long as the inter-droplet transfer of the triglyceride remained viable (Fig. 2e).

Given that lipase-mediated hydrolysis of the globules resulted in a decrease in droplet density such that the oil droplets became buoyant in the aqueous suspension, we placed a floating reservoir of tributyrin/PDMS on top of the emulsion suspension to produced droplet populations capable of temperature-dependent reversible vertical movement. At ~ 0 $^{\circ}$ C, fluorescence microscopy monitoring of buoyant droplets located directly adjacent to the oil/water interface showed slow but sustainable growth due to net uptake of tributyrin (Fig. 2f-g, Movie S4). To determine the relative rates of growth and decay of the oil droplets at various temperatures, we prepared aqueous

suspensions of lipase-coated oil droplets in the presence of excess tributyrin (solubility, 0.133 mg/mL) and monitored the change in droplet diameter under different conditions (Fig. S16-17, Movie S5). As reconstitution of the atrophied droplets was dependent on the net exchange associated with the balance between the rate of substrate uptake and the rate of lipase-mediated consumption, the extent of growth was readily moderated by changes in temperature (Fig. 2h). The maximum growth rate was observed at ~ 0 °C with a progressive decrease in expansion up to a temperature of ~ 14 °C, after which enzyme-mediated hydrolysis became increasingly dominant. The processes became balanced at ~ 17 °C to produce a steady state in which there was only minimal fluctuations in the size and reactivity of the oil droplets. At higher temperatures, the droplets continued to atrophy even in the presence of the excess tributyrin.

Cycling the temperature between 0 °C and 25 °C produced corresponding oscillations in the growth and decay of the droplets due to temperature-induced changes in the relative rates of triglyceride uptake and consumption (Fig. 2i). The oscillations were coupled to concomitant changes in buoyancy to produce populations of enzymatically active droplets capable of reversible vertical migration (Fig. S18). Typically, at 25 °C the droplets ascended in the water column and floated at the oil/water interface within 20 min (Fig. S18a-f, Movie S6). Decreasing the temperature to 0 °C inhibited the hydrolysis reaction, promoted a net uptake of tributyrin and increased the density of the floating atrophied droplets such that the droplets slowly descended to the bottom of the aqueous solution over periods greater than 190 min (Fig. S18-19, Movie S7). Thus, by switching the temperature between 0 and 25 °C, populations of the lipase-active oil droplets could be reversibly cycled up and down the water column depending on the mass transfer balance between triglyceride uptake and efflux of the water-soluble reaction products (Fig. S20).

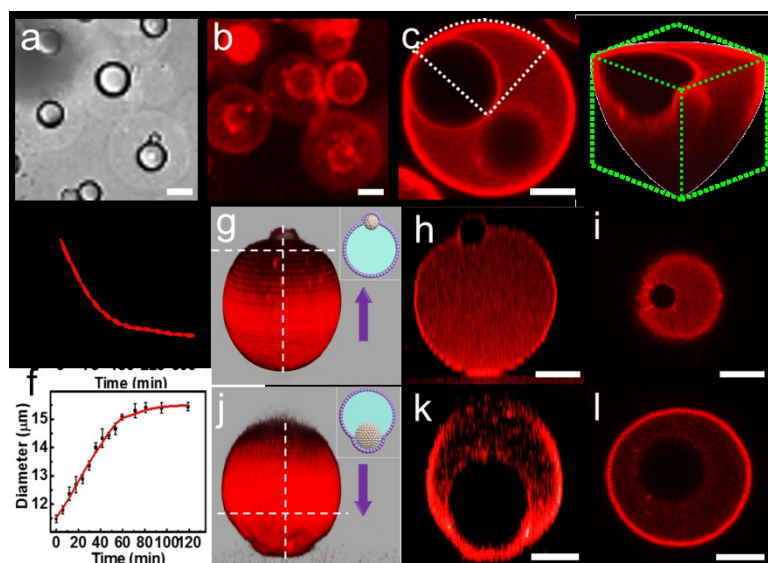


Figure 3. Optical (a) and fluorescence (b) microscopy images recorded from an aqueous dispersion of water-filled BSA-PNIPAAm proteinosomes containing encapsulated RBITC-labelled lipase-coated lipid droplets. (c,d) Corresponding confocal (c) and 3D confocal (d) fluorescence microscopy images of an individual lipid droplet-containing proteinosome. The encapsulated lipid droplet (black region) is stabilized within the proteinosomes by a continuous lipase membrane (red fluorescence). Soluble RBITC-labelled lipase is also present within the water-filled proteinosome interior. (e) Plot showing time-dependent decrease in the average diameter of a proteinosome-encapsulated lipase-coated lipid droplet (dashed yellow circle in Fig S22a-c). (f) Plot showing time-dependent increase in the average diameter of proteinosome-encapsulated lipase-coated lipid droplet (dashed yellow circle in Fig S22d-f). (g-l) 3D confocal fluorescence images of individual lipid droplet-containing proteinosomes exhibiting buoyant (g-i) or sedimentation properties (j-l). Cross-sectional images are recorded along the dotted lines shown in g and j, to give side (h,i) and vertical (k,l) views. The encapsulated lipase-coated lipid droplets produce local perturbations in the protein-polymer monolayer due to differences in the buoyant/sedimentation forces acting on the host proteinosome and lipid droplet “organelles”.

Given the above observations, we encapsulated the lipase-active oil droplets into the water-filled interior of synthetic protein-polymer micro-capsules (proteinosome) (Fig. S21)^[6] as a rudimentary step towards constructing a model synthetic protocell capable of reversible buoyance *via* the activation and deactivation of energy-rich sub-compartments. Oil droplets were stabilized within the protocells by addition at 0 °C of a mixture of tributyrin and PDMS (4:1 v/v) into an aqueous suspension of proteinosomes pre-loaded with encapsulated lipase solution. Shaking the samples intensely for 10 s resulted in incarceration of single or up to five oil droplets within the proteinosomes (Fig 3a,b), indicating that triglyceride molecules and PDMS were readily transferred across the semi-permeable chemically cross-linked

protein-polymer membrane. Confocal fluorescence microscopy images confirmed that the proteinosomes remained intact and that lipase was present at the surface of the triglyceride droplets (Fig. 3c,d). The lipase-coated lipid “organelles” did not aggregate and were free to move within the proteinosome interior. Raising the temperature to 25 °C resulted in progressive shrinking of the encapsulated oil droplets (Fig. 3e, Fig. S22), whilst decreasing the temperature to 0 °C in the presence of triacetin (a more effective feedstock than tributyrin due to increased water solubility (60 mg/mL)) in the external aqueous phase reconstituted the incarcerated droplets with additional triglyceride (Fig. 3f, Fig. S22, Movie S8-9). As a consequence, temperature-dependent changes in droplet size could be coupled to concomitant changes in the buoyancy of the entrapped droplets, which in turn resulted in reversible vertical migration of the proteinosome population (Fig. S23, Movie S10-11). Confocal fluorescence images of proteinosomes containing entrapped lipase-active oil droplets indicated that the atrophied or reconstituted sub-compartments were spatially localized against the inner surface of the intact protocell membrane (Fig. 3g-l). Moreover, local protrusions in the monomolecular protein-polymer membrane were observed in side-view cross-sections of the proteinosomes (Fig. 3g,j), consistent with a relatively large mis-match between the buoyant or sedimentation forces acting on both the proteinosomes and encapsulated oil droplets. As a consequence, vertical movement of the proteinosomes was correlated to the internal state of the enzymatically active “organelles”.

In summary, enzymatically active oil droplets were prepared *via* the spontaneous assembly of lipase at the oil droplet/water interface to produce stabilized tributyrin/PDMS globules consisting of a core-shell arrangement of substrate and biocatalyst, respectively. By employing lipase as a reactive building block, and a liquid core comprising a short-chain triglyceride and low density PDMS, *in situ* hydrolysis of tributyrin could be controlled to produce substrate-depleted droplets with tunable levels of atrophy and buoyancy. Reconstitution of the atrophied droplets was achieved by sequestration of additional feedstock *via* inter-droplet exchange or direct solution transfer of tributyrin. As a consequence, cycles of consumption and

reconstitution could be established by judicious changes in temperature to control the extent of substrate depletion and uptake, respectively. In turn, the cycles in droplet density could be correlated with reversible changes in buoyancy to produce temperature-guided reversible changes in vertical motility. These results highlight the potential for developing self-fueled oil droplets to generate potentially programmable autonomic behaviors that could be used to produce smart liquid dispersions with possible applications as active microscale objects for controlled catalysis, storage and release.

Finally, we demonstrate how lipase-coated tributyrin/PDMS droplets can be incorporated into a model protocell as a step towards constructing proteinosomes with discrete energy-rich “organelles”. The encapsulated droplets act as sub-compartments for activating protocell buoyancy as well as acidifying the micro-compartmentalized interior. In the longer term, coupling such protocells to specific chemical and temperature gradients in the local environment could provide a novel approach for producing consortia of protocells exhibiting rudimentary forms of internally generated signaling, selective motility and buoyancy-based physical sorting.

Acknowledgements

We thank NSFC (51703043, 21871069) and NSF of Heilongjiang province (F2018014) for financial support.

Keywords: • self-assembly • self-oscillating • protocells • dynamic behaviors

References:

- [1] a) S. Mann, *Acc. Chem. Res.* **2012**, *45*, 2131; b) S. Rasmussen, L. Chen, D. Deamer, D. Krakauer, N. Packard, P. Stadler, M. Bedau, *Science* **2004**, *303*, 963; c) W. Spoelstra, S. Deshpande, C. Dekker, *Curr. Opin. Biotechnol.* **2018**, *51*, 47; d) T. Beneyton, D. Krafft, C. Bednarz, C. Kleineberg, C. Woelfer, I. Ivanov, T. Vidaković-Koch, K. Sundmacher, J. Baret, *Nat. Commun.* **2018**, *9*, 2391; e) P. Luisi, *Chemistry & Biodiversity* **2012**, *9*, 2635-2647.
- [2] a) K. Morigaki, P. Walde, *Curr. Opin. Colloid Interface Sci.* **2007**, *12*, 75-80; b) F. Caschera, V. Noireaux, *Curr. Opin. Chem. Biol.* **2014**, *22*, 85-91.
- [3] J. Hindley, Y. Elani, C. McGilvery, S. Ali, C. Bevan, R. Law, O. Ces, *Nat. Commun.* **2018**, *9*, 1093.

-
- [4] a) B. Buddingh', J. van Hest, *Acc. Chem. Res.* **2017**, *50*, 769-777; b) D. Hammer, N. Kamat, *FEBS Lett.* **2012**, *586*, 2882-2890; c) J. Gaitzsch, X. Huang, B. Voit, *Chem. Rev.* **2015**, *116*, 1053-1093.
- [5] M. Li, R. Harbron, J. Weaver, B. Binks, S. Mann, *Nat. Chem.* **2013**, *5*, 529-536.
- [6] X. Huang, M. Li, D. Green, D. Williams, A. Patil, S. Mann, *Nat. Commun.* **2013**, *4*, 2239.
- [7] S. Koga, D. Williams, A. Perriman, S. Mann, *Nat. Chem.* **2011**, *3*, 720.
- [8] a) C. Martino, S. Kim, L. Horsfall, A. Abbaspourrad, S. Rosser, J. Cooper, D. Weitz, *Angew. Chem. Int. Ed.* **2012**, *51*, 6416-6420; b) N.-N. Deng, M. Vibhute, L. Zheng, H. Zhao, M. Yelleswarapu, W. Huck, *J. Am. Chem. Soc.* **2018**, *140*, 7399-7402.
- [9] C. Strulson, R. Molden, C. Keating, P. Bevilacqua, *Nat. Chem.* **2012**, *4*, 941-946.
- [10] N. Martin, M. Li, S. Mann, *Langmuir* **2016**, *32*, 5881-5889.
- [11] a) M. Marguet, C. Bonduelle, S. Lecommandoux, *Chem. Soc. Rev.* **2013**, *42*, 512-529; b) B. Städler, A. Price, A. Zelikin, *Adv. Funct. Mater.* **2011**, *21*, 14-28.
- [12] M. Hardy, J. Yang, J. Selimkhanov, C. Cole, L. Tsimring, N. Devaraj, *Proc. Natl. Acad. Sci. USA* **2015**, *112*, 8187-8192.
- [13] L. Rodríguez-Arco, M. Li, S. Mann, *Nat. Mater.* **2017**, *16*, 857-863.
- [14] Y. Qiao, M. Li, R. Booth, S. Mann, *Nat. Chem.* **2017**, *9*, 110-119.
- [15] a) Y. Yin, L. Niu, X. Zhu, M. Zhao, Z. Zhang, S. Mann, D. Liang, *Nat. Commun.* **2016**, *7*, 10658; b) S. Semenov and W. Huck *et.al. Nat. Chem.*, **2015**, *7*, 160-165.
- [16] a) P. Vonmont-Bachmann, P. Walde, P. Luisi, *J. Liposome Res.* **1994**, *4*, 1135-1158; b) H Zepik, E. Blöchliger, P. Luisi, *Angew. Chem. Int. Ed.* **2001**, *40*, 199-202; c) P. Torre, C. Keating, S. Mansy, *Langmuir* **2014**, *30*, 5695-5699.
- [17] a) J. Čejková, T. Banno, M. Hanczyc, F. Štěpánek, *Artificial Life* **2017**, *23*, 528-549; b) M. Li, M. Brinkmann, I. Pagonabarraga, R. Seemann, J. Fleury, *Commun. Phys.* **2018**, *1*, 23; c) R. Tenno, Y. Gunjima, M. Yoshii, H. Kitahata, J. Gorecki, N. Suematsu, S. Nakata, *J. Phys. Chem. B* **2018**, *122*, 2610-2615.
- [18] a) D. Zwicker, R. Seyboldt, C. Weber, A. Hyman, F. Jülicher, *Nat. Phys.* **2017**, *13*, 408-413; b) J. Taylor, S. Eghtesadi, L. Points, T. Liu, L. Cronin, *Nat. Commun.* **2017**, *8*, 237.
- [19] F. Caschera, S. Rasmussen, M. Hanczyc, *ChemPlusChem* **2013**, *78*, 52-54.
- [20] C. Jin, C. Krüger, C. Maass, *Proc. Natl. Acad. Sci. USA* **2017**, *114*, 5089-5094.

Table of Contents (Layout 1)

Autonomic Behavior in Lipase-active Oil Droplets

Lei Wang, Youping Lin, Yuting Zhou, Hui Xie, Jianmin Song, Mei Li, Yudong Huang, Xin Huang and Stephen Mann**

A new protocell model based on enzyme-powered oil droplets capable of self-consumption, feedstock regeneration, oscillatory temperature-guided contraction and reversible buoyancy-induced motility is described as a step towards autonomic behavior in life-like microscale objects.

